EXHIBIT 3

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Intrachromosomal Triplication of 2q11.2-q21 in a Severely Malformed Infant: Case Report and Review of Triplications and Their Possible Mechanism

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A female fetus with brain malformations, multicystic kidneys, absence of the right thumb, and a posterior cleft of palate was delivered at 32 weeks of gestation. Cytogenetic studies including FISH showed a novel intrachromosomal triplication of the proximal long arm of chromosome 2 (q11.2-q21), resulting in tetrasomy for this segment. The middle repeat was inverted. At least 11 patients with intrachromosomal triplications have been reported, mostly involving chromosome 15q. The mechanism involved in formation of these rearrangements is compatible with U-type exchange events among three chromatids. Am. J. Med. Genet. 82:312–317, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS: chromosome 2; intrachromosomal triplication; partial tetrasomy; 2q11.2-q21

INTRODUCTION

Intrachromosomal triplications producing partial tetrasomies are rare but have been reported for five different chromosomes. We describe an infant with cerebral, renal, and digital malformations associated with a previously unreported intrachromosomal triplication involving the proximal long arm of chromosome 2.

CLINICAL REPORT

A 32-week female fetus in double footling breech presentation was delivered by C-section to a 27-year-old.

Fig. 1. Malformed female fetus at autopsy with Potter facies caused by oligohydramnios. The right thumb is absent, although it is not well demonstrated in this view.

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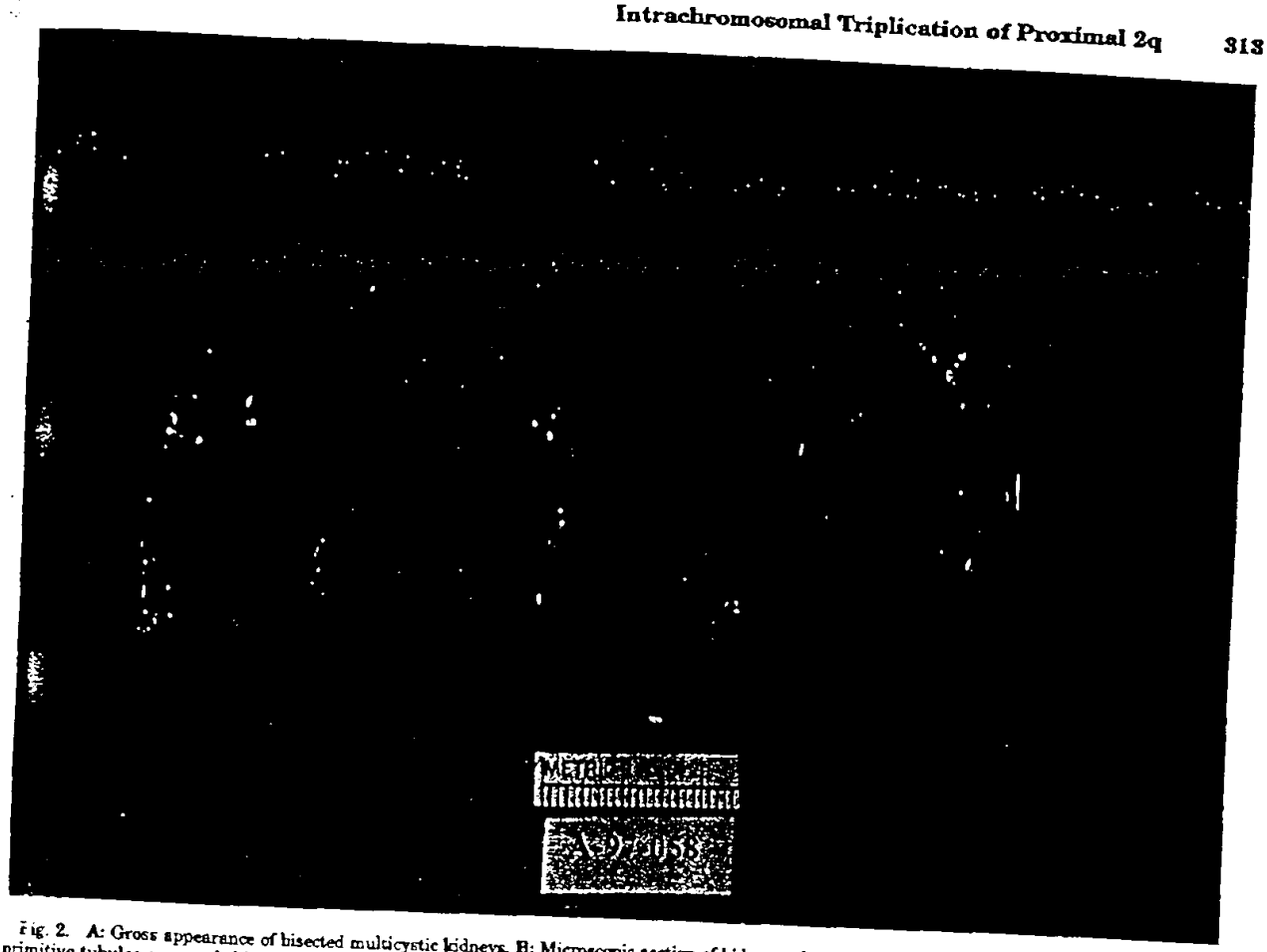

Fig. 2. A: Gross appearance of bisected multicystic kidneys. B: Microscopic section of kidneys, showing varying sized cysts lined by epithelium and primitive tubules surrounded by mesenchyme. A few clusters of glomeruli are present. Masson trichrome stain; original magnification, ×100.

gravida 6 para 5 mother. The parents were nonconsanguineous, and all five sibs were healthy with no observable anomalies. The mother reported prenatal care and denied exposure to smoking, alcohol, or drugs during pregnancy. She complained of lower abdominal pain and no fetal movement on the day of delivery. Ultrasonography showed no amniotic fluid, a large head, large, multicystic kidneys, no visible bladder, and no fetal movement. The heart rate was 50 at birth (with no spontaneous respirations and no movement), and the baby expired shortly after.

At autopsy, the weight was 1,700 g (expected 1,413 ± 623 g) [Stocker and Dehner, 1992], crown-heel length 38 cm (expected 39.8 ± 5.4 cm), and crown-rump length 26 cm (expected 28.4 ± 2.8 cm). The OFC was 34.5 cm (expected 29.5 ± 2.5 cm) with chest circumference of 24.5 cm (expected 25 ± 2.5 cm; Fig. 1). A posterior cleft of palate and webbing of the neck were present. There was absence of the right thumb, hypoplasia of the middle phalanx of the 5th finger, a single palmar crease, and clubfeet. The clitoris appeared enlarged but otherwise normal.

Internal examination showed the esophagus and trachea to be patent. Lungs displayed the usual lobe pattern but were hypoplastic (8.9 g combined; expected 25 ± 11 g combined). The heart was 9.8 g (expected 9.1 ± 4.1 g) with normal chambers and vessels. The diaphragm was intact. The kidneys were located in the appropriate retroperitoneal flank areas but were multicystic and enlarged to roughly 7 × 4 × 3 cm (Fig. 2A; 69.8 g combined; expected 12.6 ± 8.0 g combined). Renal cysts varied in diameter from a few millimeters to 3 cm and had gelatinous contents. The ureters were judged to be atretic, and the bladder was hypoplastic. The liver was architecturally normal with no cysts on sectioning (69.7 g; expected 52 ± 32 g). The stomach, small and large bowel, ovaries, uterus, and vagina had no gross abnormalities.

The brain weighed 150 g (expected 196 ± 92 g); 230 ml of clear intracranial cerebrospinal fluid was present within the dura when it was opened. The cerebral ventricles were enlarged. The cerebral cortex was thin and smooth without the gyral indentations expected at 32 weeks of gestation but with the intrahemispheric, central, and Sylvian fissures well formed. Agenesis of the septum pellucidum between the lateral ventricles, agenesis of the corpus callosum, hypoplasia of the cerebellar vermis and hemispheres, and a thin-walled, 4th ventricle cyst enveloping the brainstem (typical of a Dandy-Walker malformation with partial agenesis of the vermis) were observed. The spinal cord was unremarkable, with no anomalies of the spine.

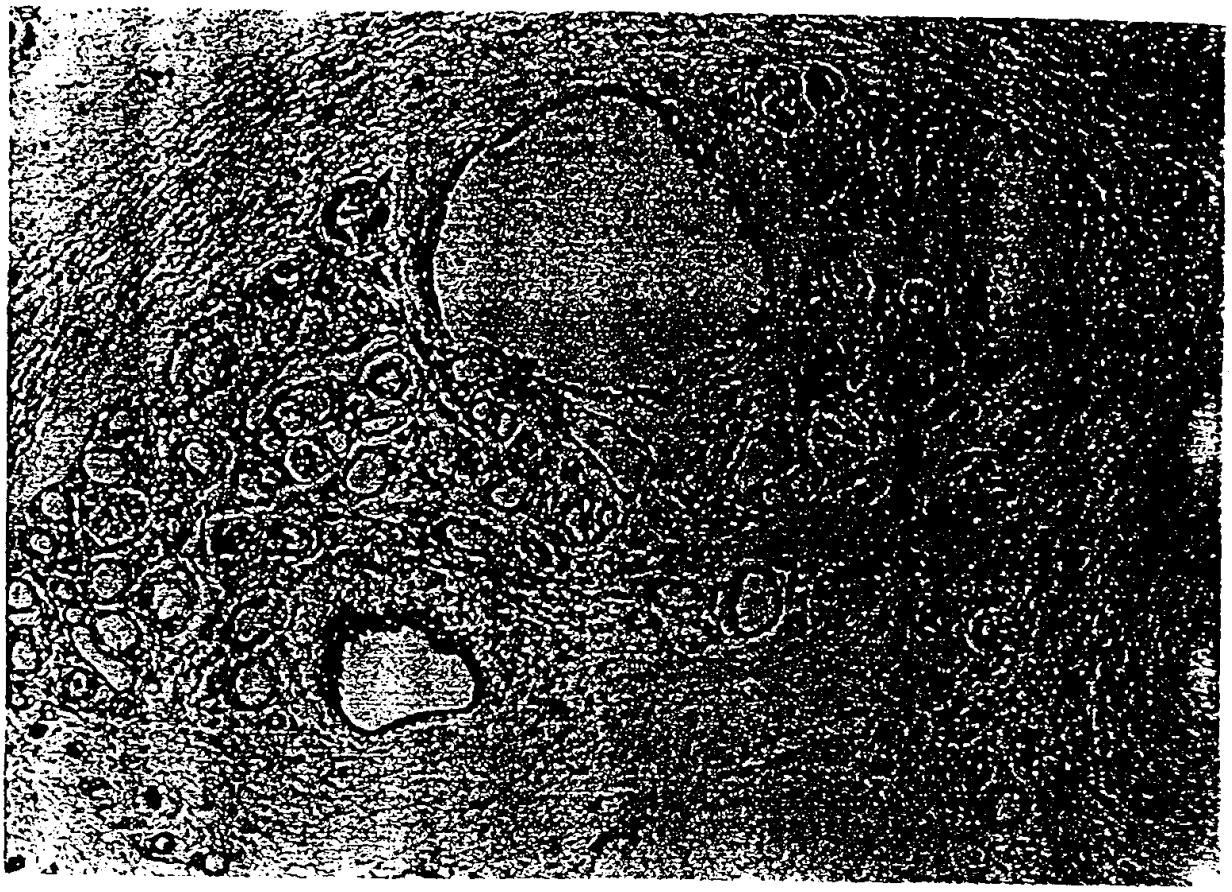

Fig. 2. (*Continued.*)

The placenta (300 g; expected 362 ± 79 g) [Singer et al., 1991] had numerous, small, round, shiny, 2 to 5 mm nodules on the amniotic surface consistent with amnion nodosum associated with oligohydramnios.

Histological examination of the kidneys showed cortical, epithelial-lined cysts and primitive tubules surrounded by mesenchyme with isolated clusters of glomeruli (Fig. 2B). The hypoplastic lungs had unexpanded alveoli and normal-appearing blood vessels. Brain sections showed poorly developed cortex with essentially complete neuron migration but no gyral formation. The cerebellar cyst wall contained disorganized neuroglial elements and vessels in a thin lamina extending around the pons and medulla. Sections of the thymus, heart, liver, spleen, pancreas, ovaries, and adrenal glands showed no histological abnormalities.

RADIOGRAPHIC FINDINGS

Anteroposterior and lateral full body films showed very significant macrocrania relative to body size. Bilateral dislocated hips were present. There were 12 ribs. Views of the right hand showed an absent thumb and hypoplasia of the 5th middle phalanx (i.e., unilateral 5th finger clinodactyly). No other significant abnormalities were observed.

CYTOGENETIC STUDIES

Long-term cultures of fetal lung tissue obtained at autopsy were carried out. Fibroblasts were harvested 30 min after addition of colcemid (0.025 μg/ml; Life Technologies, Gaithersburg, MD). Chromosomes were GTG banded. All metaphases studied had an abnormal chromosome 2. Segment q11.2-q21 was triplicated (Fig. 3A) with the middle repeat inverted based on inspection of the banding pattern.

FISH analyses were performed by use of a chromosome 2 painting probe (Ventana Medical Systems, Tucson, AZ). Chromosome preparations on a glass slide were denatured in 70% formamide/2× SSC at 70°C for 2 min followed by dehydration in 70, 80, 90%, and absolute alcohol. Ten microliters of the probe were applied to the slide, and the slide was coverslipped and sealed. Following overnight incubation at 37°C in a humid chamber, the slide was washed in 1× SSC at 72°C for 5 min. Detection was with rhodamine-labeled anti-digoxigenin and 4,6-diamido-2-phenylindole (DAPI) as the counterstain. Both chromosome 2s were painted uniformly from end to end (Fig. 3B). Uniform painting confirmed that all of the additional material was derived from chromosome 2 and not from another chromosome.

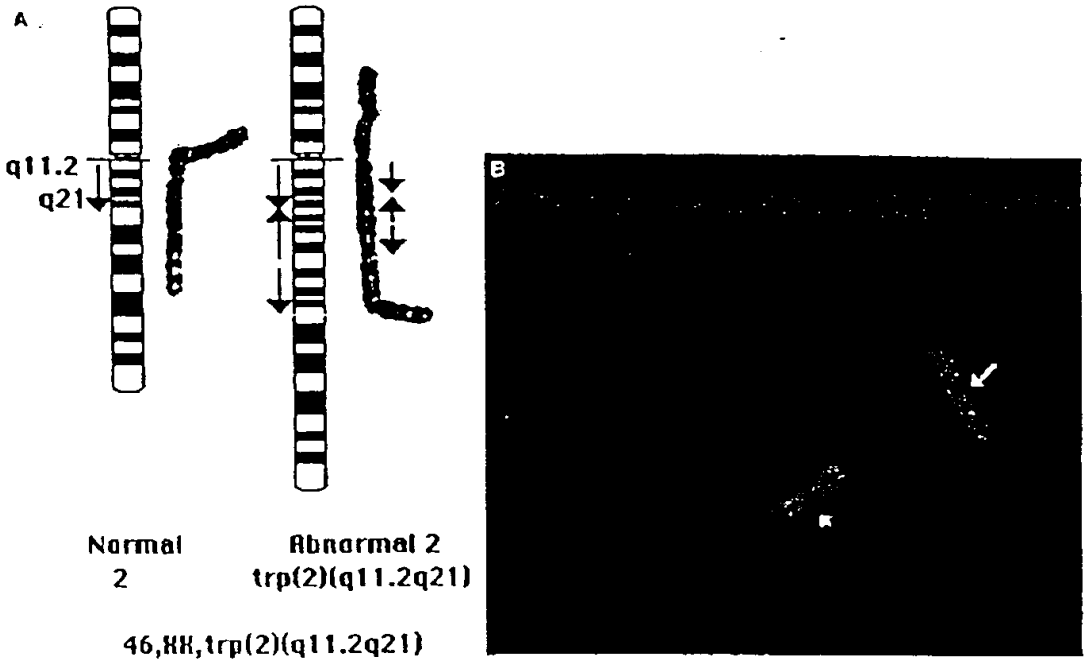

Fig. 3. A: GTG-banded chromosome 2 pair. The left image shows a normal chromosome 2 and the normal q11.2-q21 segment. The right image shows an abnormal chromosome 2 with triplicated q11.2-q21 (arrows indicate directions of the repeated segments). B: FISH study using a chromosome 2 painting probe. The normal chromosome 2 (arrowhead) and the abnormal chromosome 2 (long arrow) are uniformly painted.

The parents declined to provide blood samples for chromosome or DNA studies.

DISCUSSION

Partial duplications of the proximal long arm of chromosome 2 have been reported previously. Mu et al. [1984] described a 3-year-old girl with direct duplication of q11.2-q14.2. She had developmental delay, short stature, microcephaly, and congenital glaucoma. Cooke et al. [1995] reported a 7-year-old boy with direct duplication of q11.2-q21. The phenotype included developmental delay with ear and genital anomalies. Glass et al. [1998] observed a 37-year-old woman and her 66-year-old mother with partial trisomy for q11.2-q21.1 resulting from an insertion into chromosome 8. Both had short stature with mental retardation and were treated for a psychotic disorder.

In contrast, this case shows that triplication of the proximal long arm (partial tetrasomy) of chromosome 2 (q11.2-q21) is associated with a more severe, lethal condition. The combination of midline brain malformations, dysplastic kidneys, a radial ray malformation, and cleft palate is compatible with involvement of the midline during blastogenesis [Opitz, 1993; Martinez-Frías and Frías, 1997].

At least 11 cases of intrachromosomal triplications have been reported since 1993 (Table I). Seven of the 11 cases involved chromosome region 15q11-q13, supporting the interpretation that 15q is prone to this kind of rearrangement. At least six of the triplications, involving 2q, 5p, 7p, and 15q, had inversion of the middle repeat, suggesting a similar mechanism of origin during meiosis. Parental chromosomes were analyzed in six cases and were reported to be normal. In the seven cases in which the parental origin of the abnormal chromosome was assessed, the triplicated chromosome was maternally derived in six, and one was paternal. Preponderance of maternal triplications may be because of the longer prophase 1 of oogenesis.

Schinzel et al. [1994] suggested that intrachromosomal triplication can arise from a dicentric, inverted duplication chromosome. Breakage of the dicentric chromosome and recombination with a normal chromosome could lead to triplication. More complex mechanisms involving multiple recombinations or parental rearrangements have also been hypothesized [Rivera et al., 1998; Long et al., 1998].

An intrachromosomal triplication with an inverted middle repeat may also arise from mechanisms involving U-type exchanges among three chromatids. The exchanges may involve homologous and sister chromatids (Fig. 4A) or homologous chromatids only (Fig. 4B). U-type exchanges involving only homologous chromatids could form a dicentric chromosome as an intermediate (Fig. 4B). The triplication chromosome should contain DNA polymorphisms from both homologous chromosomes, because each contributes either one or two segments to the triplication by this mechanism. However, Harrison et al. [1998] showed evidence for a rearranged chromosome with DNA polymorphisms derived from a single chromosome without markers from its homologue. Therefore, exchanges among three chromatids may not be the only way in which triplication occurs.

In their patient with triplication of 15q11-q13, Long et al. [1998] found a supernumerary bisatellited, dicentric marker chromosome shown to be an inv dup(15)

TABLE I. Summary of Reported Cases With Intrachromosomal Triplication*

Case	Triplicated segment	Age of patient	Middle repeat inverted	Additional abnormality	Parental chromosomes	Parental origin	Reference
1	2q37	15 years	—	No	Normal	—	Rauch et al., 1996
2	5p14-p15.33	Newborn	Yes	No	Normal	Maternal (1)	Harrison et al., 1998
3	7p21.3-p22	2 years	Yes	No	Normal	—	Rivera et al., 1998
4	9p22-pter	Infant	—	idic (9)	Normal	—	Batanian et al., 1994
5	15q11-q13	2 4/12 years	—	No	—	Maternal (2)	Holowinsky et al., 1993
6	15q11-q13	7 months	—	No	—	Maternal (2)	Holowinsky et al., 1993
7	15q11-q13	7 years	Yes	No	Normal	Maternal (2)	Schinzel et al., 1994
8	15q11-q13	40 years	Yes	No	—	—	Crawford et al., 1995
9	15q11-q13	17 years	—	No	—	Maternal (–)	Chadwick et al., 1996
10	15q11-q13	6 years	—	No	—	Paternal (2)	Cassidy et al., 1996
11	15q11-q13	4 years	Yes	inv dup (15)	Normal	Maternal (2)	Long et al., 1998
12	2q11.2-q21	Newborn	Yes	No	Not tested	—	This report

*"—" indicates information was not reported. The 9p22-pter triplication in case 4 was reported as "an inversion triplication," without specific description of which segment was inverted. Parental origin with a (2) indicates that both maternal or both paternal alleles were involved in the triplication, whereas "maternal (1)" indicates that only 1 maternal allele was detected.

with a break point proximal to the locus for small nuclear ribonucleoprotein-associated polypeptide N (*SNRPN*). The partially triplicated chromosome 15 and the dicentric chromosome were seen in all cells examined. Both of our proposed mechanisms can produce a dicentric chromosome as a reciprocal product. Therefore, the reported 15q triplication and associated inv dup(15) are compatible with the 3-chromatid exchange models followed by cosegregation of two rearranged chromosomes (Fig. 4).

The 3-chromatid exchange mechanism was proposed earlier for a *Drosophila* triplication [Slizynska, 1968]. The triplication was induced by mustard gas and also contained an inverted middle repeat. The original

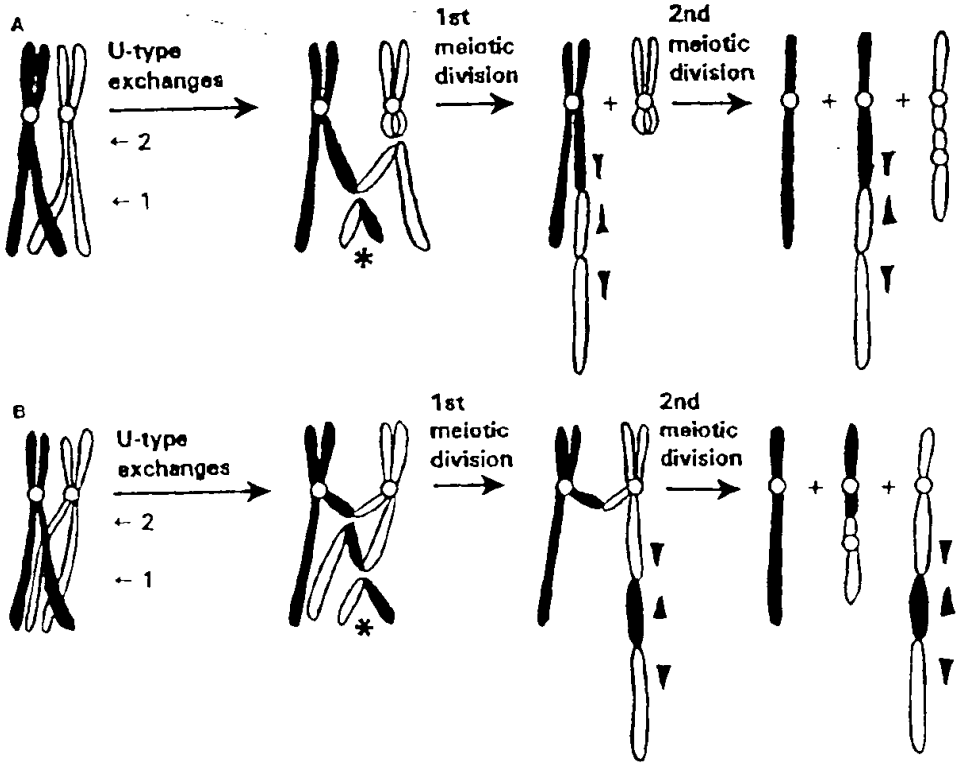

Fig. 4. Models for intrachromosomal triplications. Diagrams show U-type exchange events involving bivalent chromosomes and the resulting chromosome structures. Chromosomes with triplications have arrowheads alongside, which indicate orientations of the triplicated segments. Acentric chromosomes, marked with asterisks (*), are presumed to be lost at the next cell division. Cosegregation of rearranged chromosomes containing centromeres could occur in either model. However, we have not shown the various possible outcomes of chromosome segregation in these diagrams. A: Model with U-type exchanges involving homologous (1) and sister chromatids (2). B: Model with U-type exchanges (1 and 2) involving homologous chromatids.

model assumed that chemical mutagenesis caused "opening and rejoining of latent breaks" in chromosomes.

In summary, intrachromosomal triplications have now been observed for at least five different chromosomes. A 3-chromatid exchange mechanism may explain formation of triplications as well as associated chromosome abnormalities occasionally found in these patients.

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REFERENCES

Bekheirnia R, Chen X, Grange DK. 1994. Mosaic isodicentric chromosome 9 with triplication (9p22-pter) and no deletion in an abnormal infant presenting with clinical features of trisomy 9: a new type of isodicentric chromosome formation. Am J Hum Genet 55:A98.

Cassidy SB, Conroy J, Becker L, Schwartz S. 1996. Paternal triplication of 15q11-q13 in a hypotonic, developmentally delayed child without Prader-Willi or Angelman syndrome. Am J Med Genet 62:205–212.

Chadwick D, Strasberg PM, Farrell S. 1996. Intrachromosomal triplication of proximal 15q. Am J Hum Genet 59:A114.

Cooke LB, Richards H, Lunt PW, Burvill-Holmes L, Howell KT, McDermott A. 1995. Duplication 2 (q11.2-q21): a previously unreported abnormality. J Med Genet 32:825–826.

Crawford EC, Lethco EA, Bealer D, Schroer RJ, Clarkson KB, Phelan MC. 1995. Interstitial duplication and triplication of 15q11-q13 confirmed by fluorescence in situ hybridization. Am J Hum Genet 57:A111.

Glass IA, Stormer P, Oei PTSP, Hacking E, Cotter PD. 1998. Trisomy 2q11.26→q21.1 resulting from an unbalanced insertion in two generations. J Med Genet 35:319–322.

Harrison KJ, Teshima IE, Silver MM, Jay V, Unger S, Robinson WP, James A, Levin A, Chitayat D. 1998. Partial tetrasomy with triplication of chromosome (5) (p14-15.33) in a patient with severe multiple congenital anomalies. Am J Med Genet 78:103–107.

Holowinsky S, Black SH, Howard-Peebles PN, Mutirangura A, Christian S, Ledbetter DH, Reynolds J. 1993. Triplication 15q11-13 in two unrelated patients with hypotonia, cognitive delays and visual impairment. Am J Hum Genet 53:A125.

Long FL, Duckett DP, Billam LJ, Williams DK, Crolla JA. 1998. Triplication of 15q11-q13 with inv dup(15) in a female with developmental delay. J Med Genet 35:425–428.

Martinez-Frías ML, Frías JL. 1997. Primary developmental field III: Clinical and epidemiological study of blastogenetic anomalies and their relationship to different MCA patterns. Am J Med Genet 70:11–15.

Mu Y, Van Dyke DL, Weiss L, Olgac S. 1984. De novo direct tandem duplication of the proximal long arm of chromosome 2: 46, XX, dir dup(2Xq11.2q14.2). J Med Genet 21:57–58.

Opitz JM. 1993. Blastogenesis and the "primary field" in human development. In: Opitz JM, Paul NW, editors. Blastogenesis: normal and abnormal. New York: Wiley-Liss. p 3–37.

Rauch A, Pfeiffer RA, Trautmann U. 1996. Deletion or triplication of the α3(VI) collagen gene in three patients with 2q37 chromosome aberrations and symptoms of collagen-related disorders. Clin Genet 49:279–285.

Rivera H, Bobadilla L, Rolon A, Kunz J, Crolla JA. 1998. Intrachromosomal triplication of distal 7p. J Med Genet 35:78–80.

Schinzel AA, Brecevic L, Bernasconi F, Binkert F, Berthet F, Wuilloud A, Robinson WP. 1994. Intrachromosomal triplication of 15q11-q13. J Med Genet 31:798–803.

Singer DB, Sung CJ, Wigglesworth JS. 1991. Fetal growth and maturation: With standards for body and organ development. In: Wigglesworth JS, Singer DB, editors. Textbook of Fetal and Perinatal Pathology. Boston: Blackwell Scientific Publications. p 11–47.

Slizynska H. 1968. Triplications and the problem of non-homologous crossing-over. Genet Res 11:201–208.

Stocker JT, Dehner LP. 1992. Pediatric Pathology. Philadelphia: J.B. Lippincott Company. p 1298–1299.